REMARKS

The Office Action stated that claims 1-11, 14-27, and 34-35 are pending in the instant application. However, Applicants' Amendment and Response filed July 17, 2001, which the Examiner has acknowledged (Paper No. 14), added new claims **33**-35. Therefore, claims 1-11, 14-27 and **33**-35 are pending in this application.

Applicants acknowledge the election without traverse of Group I (claims 1-27), and species of the specific complex of a TCR tetramer comprising four αβ dimers and the specific linker molecule of avidin. Thus, claims 1-11, 14-27 and **33**-35 read on the elected species.

As requested by the Examiner, the Abstract has been amended to conform with MPEP § 608.01(b) (*see*, Substitute Specification).

As requested by the Examiner, Applicants have amended the specification to list the appropriate SEQ ID NOS for the sequences disclosed in the Brief Description of the Drawings and for the sequences appearing on page 57 (*see*, Substitute Specification).

The Examiner suggested guidelines for the preferred layout of the application. The specification is amended herein to conform to the suggested guidelines.

The Examiner objected to the absence of a brief description of the several views of the Drawings. The specification is amended herein to move the brief descriptions of the drawings to immediately follow the Summary of the Invention.

Rejections under 35 U.S. C. § 103(a)

(i) The Examiner rejected claims 1-11, 14-18, and 34-35, under 35 U.S.C. § 103(a), as being unpatentable over WO 97/35991 in view of Golden *et al.*, O'Shea *et al.*, Garboczi *et al.*, and Schatz.

Specifically, the Office Action suggested that WO 97/35991 teaches soluble, recombinant divalent and multivalent analogs (including tetravalent) of heterodimeric proteins such as αβ TCR that possess enhanced affinity for their target molecules, wherein the αβ TCRs are

associated via Ig linker molecules which may further comprise a toxin and/or may be further linked by association via avidin. WO 97/35991 is also suggested to teach the production of the TCR multimers in baculovirus with a yield of 1 μg/ml; the use of short, flexible Gly-Ser spacers between the TCR chain and the Ig portion; and that multimeric soluble TCR complexes may be useful in defining the specific peptide/MHC ligands. Golden *et al.* is relied on to teach soluble heterodimeric TCR, produced in *E. coli* at yields of 4-5 mg/L, comprising α and β chain, each chain comprising a leucine zipper which dimerizes, one with the other. O'Shea *et al.* is relied upon to teach heterodimer formation through leucine zippers from c-fos and c-jun. Garboczi *et al.* is alleged to teach a soluble TCR without the interchain disulfide bond present in native TCRs, and that heterodimerization, refolding and antigenic specificity of the TCR do not require its interchain disulfide bond, trans-membrane segments or glycosylation. Finally, Schatz is relied upon to teach that biotinylation of proteins is of practical importance primarily due to the very tight binding of biotin to the proteins avidin and streptavidin, and that it is advantageous to accomplish biotinylation at a single site using an agent with site specificity.

The Office Action states that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have made a recombinant TCR as taught by the combination of WO 97/35991 and Golden *et al.* without the disulfide bond, as taught by Garboczi *et al.* and further modified as taught by Schatz. It is alleged that one of ordinary skill in the art would have been motivated to combine these references in order to increase the yield of correctly folded soluble TCR of WO 97/35991 as modified with leucine zippers taught by Golden *et al.* and O'Shea *et al.*, and because Garboczi *et al.* teach that the presence of a disulfide bond is not important for heterodimerization and refolding, and further in order to facilitate the production of soluble correctly associated TCR.

Applicants respectfully disagree with this characterization of the references, particularly of Garboczi *et al.* Applicants further note that Garboczi *et al.* is an essential element in the reasoning supporting the rejections and, unless it adequately supports the interpretation stated in the Office Action, the entire objection is unsustainable. Applicants submit that upon further review, Garboczi *et al.* do not provide motivation for one skilled in the art to omit the disulfide bond, as required by Applicants' claimed invention.

In particular, Applicants submit that the experiments reported by Garboczi *et al.* suggest that the TCR heterodimers produced by the method of Garboczi *et al.* are not stable absent the interchain disulfide bond and, therefore, teach away from the present invention.

Figure 2A of Garboczi *et al.* shows the results of experiments in which "the TCR preparations used . . . represent the disulfide-bonded long forms of the α and β subunits" (see figure legend; emphasis added). Therefore, these experiments are not relevant to the present application.

Figure 2B of Garboczi *et al.* shows the results of experiments in which "Iodoacetamide treated long forms of both subunits [were] used" (see figure legend) in polyacrylamide gel electrophoresis (PAGE) comparisons. Iodoacetamide acts to block the cysteine residues and thereby prevents disulfide bond formation. However, in none of the six lanes of the gel of Figure 2B were the iodoacetamide-treated α and β subunits tested for heterodimer formation in the absence of a stabilizing MHC molecule and an MHC-binding peptide. In particular, the only lane which includes both the α and β subunits (lane 6) also includes the MHC molecule (HLA-A2) and the MHC-binding peptide (Tax) which help to stabilize the heterodimer. Therefore, these experiments do not demonstrate that the α and β subunits of Garboczi *et al.* form a heterodimer absent the interchain disulfide bond.

Figure 3 of Garboczi *et al.* shows the results of experiments in which the disulfide-bonded TCR complex from the native gel shown in Figure 2A was subjected to PAGE to confirm that it contained both the disulfide-bonded TCR and the MHC protein (HLA-A2). Lane 1 of the gel shown in Figure 3 was run under denaturing and non-reducing conditions which cause the proteins present to unfold from their native tertiary conformations, but will not disrupt any interchain disulfide bonds. The TCR-complex, including the interchain disulfide bond, forms a distinct band at approximately 60 kDa. Lane 2 of the gel of Figure 3 was run under denaturing and reducing conditions which both cause the proteins to denature and disrupt any interchain disulfide bonds. As Garboczi *et al.* state: "Under reducing conditions (Fig. 3, lane 2) the 60-kDa band is converted to a 29-kDa band likely containing both the α- and the β-chains of the TCR" (pg. 5405, second column, second paragraph, lines 14-16). Thus, under reducing conditions which eliminate the interchain disulfide bond, Garboczi *et al.* show that the 60-kDa

TCR heterodimer dissociates into its constituent ~29-kDa chains. Therefore, these experiments suggest that the disulfide bond of Garboczi *et al.* is necessary for heterodimer formation.

Figure 4 of Garboczi *et al.* shows the results of experiments in which the short forms of the α and β subunits (i.e., truncated forms lacking the cysteines necessary for double bond formation) were subjected to electrophoresis under denaturing conditions. In lane 1, the proteins were run under non-reducing conditions. In lane 2, the proteins were run under reducing conditions (which would disrupt intrachain disulfide bonds). It is clear from both lanes of Figure 4 that no αβ TCR heterodimers were formed but, rather, that the α and β subunits formed distinct bands.

Figure 5A of Garboczi *et al.* shows the results of an experiment in which the short forms of the α and β subunits (i.e., truncated forms lacking the cysteines necessary for double bond formation) were subjected to electrophoresis under non-denaturing (native) and non-reducing conditions. In the PAGE gel shown in Figure 5A, increasing amounts of the TCR α and β chains were run with (lanes 3, 5, 7, and 9) and without (lanes 2, 4, 6, 8) the MHC molecule (HLA-A2) and MHC-binding peptide (Tax). In each case in which the TCR α and β chains were run alone (lanes 2, 4, 6, 8) it is clear that no αβ TCR heterodimers were formed but, rather, that the α and β subunits formed distinct bands. In contrast, in the lanes in which the MHC molecule (HLA-A2) and MHC-binding peptide (Tax) were present with the TCR α and β chains (lanes 3, 5, 7, and 9), it is clear that an MHC-peptide-TCR αβ complex is formed. Indeed, in none of the lanes is there a band corresponding in molecular weight to an uncomplexed TCR αβ heterodimer. Therefore, this experiment clearly suggests that an MHC molecule (HLA-A2) and MHC-binding peptide (Tax) are necessary to stabilize heterodimers between the TCR α and β chains of Garboczi *et al.*

Figures 5B and 5C show experiments similar to that in Figure 5A (short form TCR α and β chains tested under non-denaturing, non-reducing conditions). In each lane of these gels in which the TCR α and β chains are present without an MHC molecule and MHC-binding peptide (i.e., lane 2 of Figure 5B and lanes 3 and 6 of Figure 5C), it is clear that no αβ TCR heterodimers were formed but, rather, that the α and β subunits formed distinct bands.

Taken together, these results show that the TCR α and β chains of Garboczi *et al.* without interchain disulfide bonds ***do not form*** heterodimeric TCRs except when held in a complex with an MHC molecule and MHC-binding peptide. Therefore, the suggestion in the Office Action that the teachings of Garboczi *et al.* indicate that the disulfide bond is not important for the stability of soluble TCRs is incorrect. Garboczi *et al.* simply do not provide any experimental support for such a conclusion. Thus, a person of ordinary skill in the art would not use the teaching of Garboczi *et al.* to arrive at the present invention.

The skilled person would also not use Golden *et al.* to arrive at the present invention because the TCR of Golden *et al.* (in which the TCR chains are disulfide-linked and have C-terminal leucine zippers) is not shown to be active, *i.e.*, it is not shown to bind to a peptide-MHC complex. In fact, the only indication of TCR formation in Golden *et al.* is that these molecules are recognized by TCR-specific antibodies. Recognition by antibodies is a completely different characteristic from the ability to bind peptide-MHC. Accordingly, Golden *et al.* provides no motivation to use a TCR with C-terminal dimerization domains to produce molecules capable of binding peptide-MHC.

To make a *prima facie* case of obviousness, the Examiner has the burden of showing either that some objective teaching in the prior art or knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references. *In re Fine*, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988). Indeed, the prior art must suggest the combination or convey to those of ordinary skill in the art a reasonable expectation of success of making it. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). The teachings of the references can be combined only if there is some suggestion or incentive to do so. ACS Hospital Systems, Inc. v. Montefiore Hospital, 221 USPQ 929, 933 (Fed. Cir. 1984). Because Garboczi *et al.* does not teach or suggest that the disulfide bond is not important to heterodimerization of TCR, there is no motivation provided by the teachings of Garboczi et al. to modify the soluble TCRs of Golden *et al.* and/or WO 97/35991 by removing the interchain disulfide bond. Furthermore, since Golden *et al.* do not provide any evidence that their TCR molecules are capable of binding peptide-MHC, there is no motivation to combine the teachings of Golden *et al.* with any of the cited references.

Therefore, Applicants respectfully submit that the grounds for this rejection have been overcome and request that this rejection be reconsidered and withdrawn.

(ii) Claims 1, 24 and 25 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 97/35991 in view of Golden *et al.*, O'Shea *et al.*, Garboczi *et al.*, Schatz and U.S. Patent No. 5,635,363.

WO 97/35991, Golden *et al.*, O'Shea *et al.*, Garboczi *et al.*, Schatz have been relied upon for the reasons of the rejection discussed above. U.S. Patent No. 5,635,363 is relied upon to disclose soluble MHC/peptide tetramers which are biotinylated and multimerized with streptavidin or with avidin and which further comprise a light detectable label or an enzyme and which may further be bound to an insoluble support such as a bead, *i.e.*, a "solid structure", for the purpose of assay.

The Office Action suggests that it would have been *prima facie* obvious to one of ordinary skill at the time the invention was made to have biotinylated the soluble TCR complexes taught by the combined references and to have multimerized them using avidin, and further to have labeled them with a detectable label. The Office Action states that one of ordinary skill in the art at the time of the invention would have been motivated to do this to form more avid multimers.

As explained above, a critical element of the two independent claims of the present invention, namely that the "disulfide bond present in native TCRs between the α and β or γ and δ chains adjacent to the cytoplasmic domain, is absent from the recombinant TCR," is simply not taught or suggested by any of the claimed references, either alone or in combination. As explained above, careful review of the experiments of Garboczi *et al.* demonstrate that this reference teaches away from removal of the disulfide bond. Thus, there is no teaching, suggestion or motivation to arrive at Applicants' claimed invention. Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

(iii) Claims 1, and 19-24 were rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 97/35991 in view of Golden *et al.*, O'Shea *et al.*, Garboczi *et al.*, and further in view of Ahmad *et al.*

The Office Action relied on WO 97/35991, Golden *et al.*, O'Shea *et al.*, and Garboczi *et al.*, as discussed earlier. Ahmad *et al.* is invoked to teach attachment of a biotinylated targeting antibody to the surface of a liposome containing biotinylated phosphatidylethanolamine by means of an avidin linker and to teach that liposomes containing lipid derivatives of polyethylene glycol have circulation times sufficiently long to allow for effective *in vivo* drug delivery.

The Office Action states that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have attached the multimeric TCR complex taught by the combined references to the liposome of Ahmad *et al.* The Action argues that the motivation to do this would arise from the need to effectively deliver *in vivo* the multimeric TCR complex taught by the combined references to be useful for *in vivo* therapy.

As explained above, since WO 97/35991, Golden *et al.*, O'Shea *et al.*, and Garboczi *et al.* do not teach, suggest, or motivate one of ordinary skill in the art to arrive at Applicants' invention, and because Ahmad *et al.* does not cure this deficiency, Applicants respectfully submit that the grounds for this rejection have been overcome. Accordingly, Applicants request reconsideration and withdrawal of this rejection.

CONCLUSION

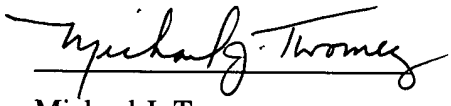
Claims 1-11, 14-27 and 33-35 were pending in the application. No claims are added, canceled or amended by the present submission.

Applicants respectfully request reconsideration of the application in light of the amendments and remarks made herein. If the Examiner believes that a telephonic interview would expedite the allowance of the application, the Examiner is invited to contact the undersigned attorney at the number below.

Applicants submit herewith a Petition for one month Extension of Time and a Request for Continued Examination. The Commissioner is hereby authorized to charge the requisite fees to Deposit Account No. 08-0219.

Respectfully submitted,
Hale and Dorr, LLP

Date: July 25, 2003



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